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Sulfoform generation from an orthogonally protected disaccharide

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ABSTRACT

An orthogonally protected disaccharide (GlcN(α1→4)Glc) with a β-linked 2′-aminoethyl linker was used to generate a series of sulfated derivatives (sulfoforms), with a 6-*O*-sulfate on the glucose residue and one or more sulfate esters on the terminal glucosamine. Deprotection and sulfonation steps were performed in solution and in variable order, with isolated yields of 36–54% (85–90% per operation) after HPLC purification. The modular deprotection–sulfonation sequences can be performed with efficient recovery of the polysulfate products, and avoids complications associated with heterogeneous reactivity in solid-phase synthesis.

1. Introduction

Proteoglycans and sulfomucins provide a number of protective and recognition functions, the latter being involved in cell adhesion, migration, proliferation, and infection.[1,2] Heparan sulfate (HS) proteoglycans and other members of this diverse family of cell-surface carbohydrates are implicated in the recruitment of growth factors and chemokines known to promote or inhibit angiogenesis,[3,4] and in the attraction of leukocytes that regulate the assembly and degradation of the extracellular matrix.[5,6] Many studies have shown that the specificity of HS-binding proteins is defined in large part by their affinity to specific sulfate patterns (sulfoforms) encoded within proteoglycans,[7,8] and the identification of such structures can be applied toward the development of new anti-inflammatory agents and immunotherapies based on glycan recognition.[9,10] Even relatively simple sulfoforms can exhibit significant influence over protein recognition and signaling pathways,[11,12] but progress in the structure–activity relationships of HS-like compounds is hampered by the difficulty of obtaining well-defined sulfoforms in sufficient quantities for further study.

In order to create libraries of sulfoforms with structures similar to those found in cell-surface glycans, we have investigated the modular deblocking and sulfonation of orthogonally protected carbohydrate derivatives. This approach enables us to generate both natural and unnatural (or unidentified) sulfoforms, as opposed to the more conventional strategy of developing precursors with pre-designated sites for sulfonation. A set of six orthogonal protecting groups for five hydroxyls and the C2 amine of a heparan disaccharide were previously identified and validated, and their cleavage was also shown to be compatible with neighboring sulfate esters.[13] To obtain fully deprotected sulfoforms, we have conducted modular deblocking-sulfonation conditions on orthogonally protected glucosamines (GlcNs) immobilized on tritylated polystyrene (PS) resins.[14,15] The solid-phase method can generate entire sets of mono- and disaccharide sulfoforms from a common precursor with up to three sulfate esters per GlcN, followed by mild cleavage and ion-exchange conditions to yield the final sulfoforms as sodium salts. We recently optimized this procedure to generate free GlcN sulfoforms in 8 operations or less with overall yields ranging from 41% (average yield per step: 90%) to 76% (average yield per step: >96%).[15] This strategy can also produce sulfoforms with 2-aminoethyl linkers, to enable their conjugation onto activated substrates or for the preparation of neoglycolipids or neoglycoproteins.

The solid-phase method of generating sulfoforms does have some limitations, however. The mild condition for cleaving the aminoethyl group from the trityl-PS resin (30% hexafluoroisopropanol in CH_2Cl_2) precludes the use of stronger acids in the orthogonal deprotection scheme. Furthermore, the efficiency of sulfonation can vary depending on the number of sulfate esters per glycan: as the charge density increases the environment within the resin becomes heterogeneous, encouraging the formation of ionic bridges within a nonpolar environment. This can have adverse effects on resin swelling and raise the barrier to chemical diffusion and exchange. Resins are also mechanically fragile and may be less well suited for reactions involving large temperature increases (due to thermal expansion of the resin), or aggressive chemical reagents that may cause polymer degradation.

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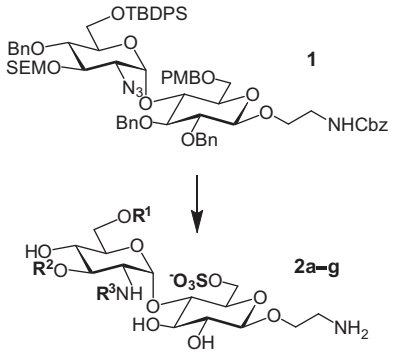


Figure 1. GlcN(α1→4)Glc sulfoforms with 2-aminoethyl linker (**2a**–**g**), generated from an orthogonally protected disaccharide (**1**). R^1,R^2 = H or SO_3^-; R^3 = Ac or SO_3^- (see Table 1).

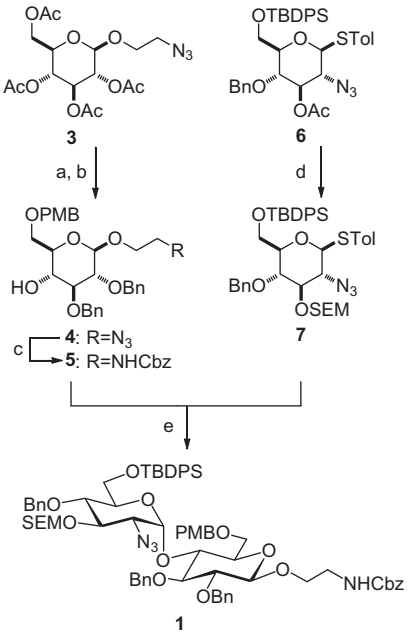
In light of these factors, we considered whether modular deblocking-sulfonation sequences might generate fully deprotected sulfoforms in solution with comparable yields and purity as those obtained using solid-phase methods. Working with charged intermediates in solution can be daunting: Most synthetic routes to sulfated oligosaccharides are designed so that sulfonation is the last step, which minimizes the number of operations needed to isolate these highly polar products. If the order of deprotection and sulfonation is variable, then some charged intermediates will be subjected to multiple processing steps. On the other hand, solution-based methods are clearly compatible with the formation of multiple sulfate esters, so long as the products are sufficiently stable to subsequent deprotection steps.

In this article we describe seven sulfoforms generated from a single, orthogonally protected disaccharide with a β-aminoethyl linker (Fig. 1), using modular deblocking-sulfonation sequences performed in solution. We focus on derivatives with a 6-*O*-sulfate ester on the reducing-end glucoside and variable sulfate patterns on the terminal glucosamine, with the latter representing the structural diversity of HS and sulfomucins. Low molecular-weight disaccharide sulfoforms (unnatural derivatives as well as natural) have been found to exhibit significant binding affinity for mucin- and HS-binding proteins such as L-selectin[16,17] and FGF-2.[11] We compare the efficiency of generating sulfoforms using solution- and solid-phase methods, and the relative benefits of reaction optimization in solution versus the expedient purification of synthetic intermediates immobilized on resins.

2. Results and discussion

2.1. Synthesis of orthogonally protected GlcN(α1→4)Glc disaccharide

2′-Azidoethyl β-glucoside tetraacetate **3**, which was prepared according to literature procedures,[15,18] was converted into glycosyl acceptor **4** in 4 steps and 61% overall yield. The azide was then converted into carboxybenzyl (Cbz) carbamate **5** in 92% yield (Scheme 1). For the reductive cleavage of the 4,6-anisylidene acetal using BH_3/Bu_2BOTf, we note that the regioselectivity is temperature-dependent, and that performing the reaction at −78 °C yields exclusively the 6-*O*-PMB ether.[19] Orthogonally protected 2-azido-2-deoxyglucose **6**, which was also prepared according to the literature procedures,[14,15] was converted into 3-*O*-SEM ether **7** in 91% yield, then coupled with **5** using *N*-iodosuccinimide (NIS)/TfOH activation to yield α-1,4-linked disaccharide **1** as the sole product in 68% yield. We also examined activation conditions using benzenesulfinyl piperidine (BSP)/Tf_2O[13,20] and phenylsulfenyl chloride (PhSCl)/AgOTf,[21] but these were found to be less effective than NIS/TfOH activation.



Scheme 1. Synthesis of orthogonally protected GlcN(α1→4)Glc derivative **1**.

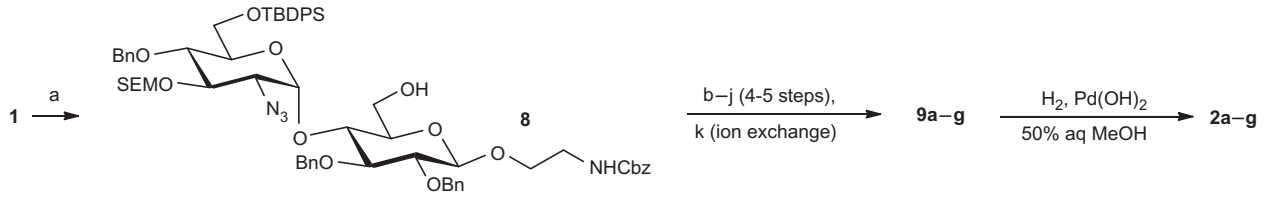
Reaction conditions: (a) (i) NaOMe, MeOH, rt; (ii) *p*-MeO(C_6H_4)-$CH(OMe)_2$, CSA, THF, reflux; (iii) BnBr, NaH, DMF, 0 °C to rt (62% over 3 steps). (b) BH_3, Bu_2BOTf, THF, −78 °C (99%). (c) (i) Bu_3P, CH_2Cl_2, then 1:1 CH_2Cl_2/H_2O, rt; (ii) Cbz-Cl, $NaHCO_3$, 5:1 THF/H_2O, 0 °C to rt (92% over 2 steps); (d) (i) NaOMe, MeOH, rt; (ii) SEM-Cl, *i*Pr_2NEt, TBAI, CH_2Cl_2, rt (91% over 2 steps). (e) NIS, TfOH, 2:1 Et_2O/$(CH_2Cl)_2$, −30 °C (68%). Selected abbreviations: Cbz = carboxybenzyl; PMB = *p*-methoxybenzyl; SEM = 2-(trimethylsilyl)ethoxymethyl; TBDPS = *tert*-butyldiphenylsilyl; Tol = *p*-tolyl.

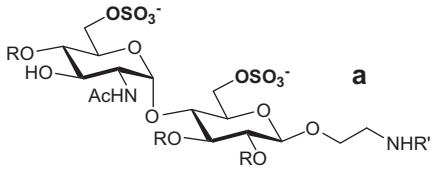
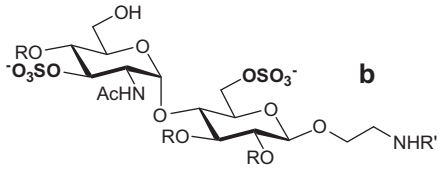
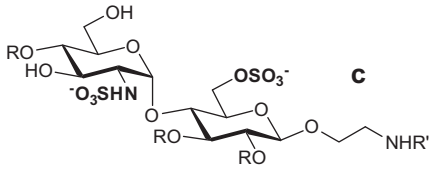
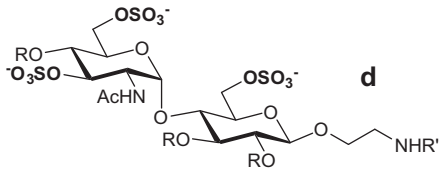
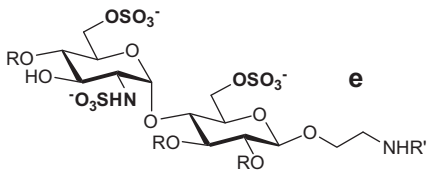
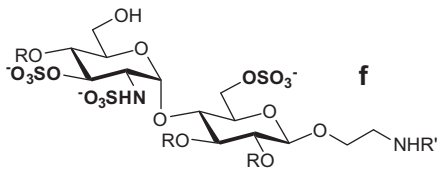
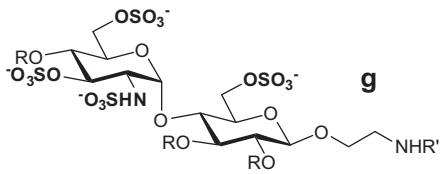
2.2. Generation of GlcN sulfoforms

We have previously developed deblocking conditions for the azide, PMB, SEM, and TBDPS groups that are fully orthogonal and also compatible with sulfate esters. [13] In this study, all derivatives have a sulfate ester at the C6 position, so the first step is to remove the PMB ether from **1** using ceric ammonium nitrate (CAN) in aqueous CH_3CN at 0 °C to afford C6 alcohol **8** in 88% yield (Table 1, step *a*). To obtain variable sulfate patterns on the 1,4-α-linked GlcN moiety, the following operations were performed in variable order: (i) TBDPS deprotection at the C6′ position using tetrabutylammonium fluoride (TBAF, step *b* or *c*), (ii) conversion of the azide at the C2′ position to –NHAc (step *d*)[22] or a free amine (step *i*),[23] (iii) removal of the SEM ether at the C3′ position using $MgBr_2$ and CH_3NO_2 (step *g* or *h*),[24] and (iv) sulfonation of the free hydroxyls and amines (step *e*, *f*, or *j*). These are followed by ion-exchange and HPLC chromatography to obtain partially benzylated sulfoforms **9a**–**g** as sodium salts (step *k*). Finally, global deprotection of the benzyl (Bn) and Cbz groups was performed under standard hydrogenation conditions to obtain aminoethyl sulfoforms **2a**–**g** in high yields. The sequence of operations and isolated yields for each sulfoform are summarized in Table 1.

With respect to TBDPS and SEM deprotections, there exist significant differences in deblocking conditions when performed before or after sulfonation. Deprotections prior to sulfonation (steps *b* and *g*) took less time (6–10 hours) than those performed after sulfonation (steps *c* and *h*: 1–2 days); the latter also required more polar solvents. TBDPS deprotections after sulfonation using TBAF

Table 1
Generation of partially and fully deprotected GlcN(α1→4)Glc sulfoforms



Sulfoform[a]	**9** (Yield from **1**, ops)[b]	**2** (Yield from **9**)[c]
 **a**	52%, 6 ops (a, b, d, e, h, k)	87%
 **b**	39%, 6 ops (a, g, d, e, c, k)	83%
 **c**	36%, 7 ops (a, e, h, c, i, j, k)	84%
 **d**	48%, 6 ops (a, b, g, d, f, k)	97%
 **e**	42%, 6 ops (a, b, i, f, h, k)	98%
 **f**	40%, 6 ops (a, g, i, f, c, k)	97%
 **g**	54%, 6 ops (a, b, g, i, f, k)	99%

[a] **9a–g**: R = Bn, R′ = Cbz; **2a–g**: R, R′ = H. All products are isolated as sodium salts.
[b] Isolated yield after HPLC purification. Each step was conducted at rt unless otherwise noted. Reagents and conditions: (a) CAN (3 equiv), 90% aq CH_3CN, 0 °C, 10 h; (b) TBAF (6 equiv), THF, 6 h; (c) TBAF (8–10 equiv), 1:1 THF/DMF, 24–40 h; (d) 1:5 AcSH/Py, 48 h; (e) $SO_3{\cdot}Me_3N$ (20–30 equiv), DMF, 55 °C, 12 h; (f) $SO_3{\cdot}Py$ (20–30 equiv), 1:5 Et_3N/Py, 70 °C, microwave, 1 h; (g) $MgBr_2{\cdot}Et_2O$ (10 equiv), CH_3NO_2 (20 equiv), Et_2O, 10 h; (h) $MgBr_2{\cdot}Et_2O$ (10 equiv), 1:2 CH_3NO_2/Et_2O, 24–48 h; (i) $HS(CH_2)_3SH$ (35 equiv), Et_3N (35 equiv), MeOH, 24 h; (j) $SO_3{\cdot}Py$ (10 equiv), 5:1 H_2O/DMF, pH 9.5, 8 h; (k) Na^+ ion-exchange chromatography.
[c] 10% $Pd(OH)_2$, H_2 (1 atm), 1:1 MeOH/H_2O, 20 h.

were also somewhat lower yielding, possibly due to complications caused by counterion exchange with nearby sulfate esters. With respect to the efficiency of sulfonation, we observed this reaction to become increasingly sluggish when attempting to install several sulfate esters under thermal conditions. We thus investigated conditions involving microwave-assisted heating, which has been used to generate carbohydrates with multiple sulfate esters.[25] A systematic variation in reaction solvent, time, and temperature revealed that microwave-assisted sulfonation is indeed more effective, but also highly temperature-sensitive. Reactions could be performed at 70 °C with minimal decomposition and generation of by-products, but reactions over 80 °C led to the rapid formation of a black tar. Sulfonation was most efficient when performed in pyridine buffered with Et_3N, which also enabled simultaneous *N*- and *O*-sulfate generation (**2e**–**g**). In comparison, the synthesis of 2′,6-di-*N*,*O*-sulfate (**2c**) under conventional thermal conditions required separate sulfonation steps.

It is worthwhile to compare the synthetic efficiency of the modular deprotection–sulfonation sequence in solution with that performed on resin supports. We recently reported the solid-phase synthesis of a similar series of α-glucosamine sulfoforms (mono- and disaccharides **10** and **11**), with overall yields ranging from 42% to 76% over 7–8 operations (Table 2).[15] These yields are higher on average than those reported in Table 1, but not uniformly so; for example, the solid-phase syntheses of 2,6-di-*N*,*O*-sulfate derivatives **10e** and **11e** are comparable to the solution synthesis of the 2′,6′,6-tri-*N*,*O*,*O*-sulfate derivative **9e**. In addition, we have encountered several practical limits with solid-phase methodologies. These limits included scalability and compound loading, the range of solvents that permit appreciable resin swelling, and the long reaction times required for the completion of each step, particularly sulfonation. We considered using microwave-assisted heating to increase the efficiency of solid-phase sulfonation; however, attempts to do so with $SO_3{\cdot}Me_3N$ in DMF at 70 °C resulted in extensive degradation of the supporting resin.

In closing, we find that orthogonal deprotection–sulfonation sequences can be performed in solution without a serious attrition in synthetic efficiency, relative to that performed by solid-phase synthesis. This process can produce aminoethyl-linked disaccharide sulfoforms in good overall yield, for subsequent conjugation onto substrates for affinity screening against heparin-binding proteins and pathogens.

3. Experimental details

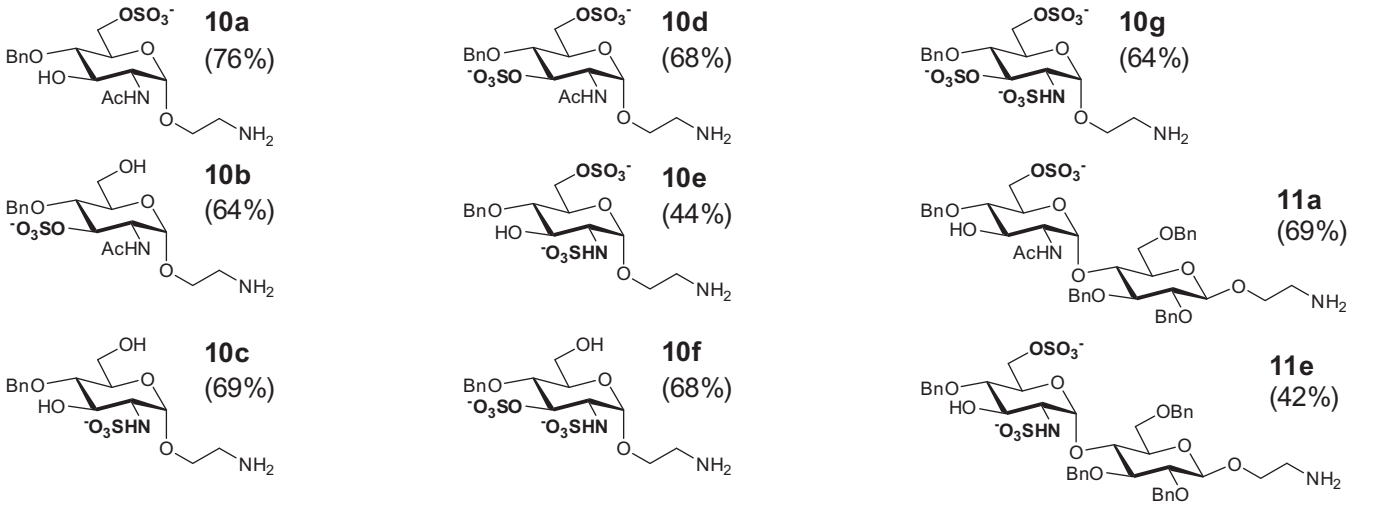
3.1. General methods

All starting materials and reagents were obtained from commercial sources and used as received unless otherwise noted. All solvents were freshly distilled prior to use. IR spectra were acquired from NaCl plate, using a Thermo-Nicolet Nexus 670 FT-IR spectrometer. ^1H and ^{13}C NMR chemical shifts were referenced to the solvent used (δ 7.27 and 77.00 for $CDCl_3$, δ 3.31 and 49.15 for CD_3OD, and δ 4.80 for D_2O at 295 K). Mass spectra were acquired using either a Hewlett-Packard 5989B or a Finnigan 40000 mass spectrometer. Optical rotations were measured by polarimetry at rt. Silica gel chromatography was performed with ICN SiliTech 32-63D. Ion-exchange chromatography was performed by dissolving sulfoforms in 50% aq MeOH (up to 100 mg/mL) and passing them through a Dowex Marathon MSC column (14 × 1.5 cm), which was activated with 0.1 M NaOH and washed with water prior to use. HPLC purifications were performed by dissolving sulfoforms in CH_3CN (up to 20 mg/mL) and injecting them onto a C18 reverse-phase column (250 × 10 mm, 4 μm beads, 80 Å pores) with UV detection at 214 nm, using a binary solvent gradient (up to 40% aq CH_3CN) with a flow rate of 5 mL/min.

3.2. 2′-Azidoethyl 2,3-di-*O*-benzyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranoside (4)

2′-Azidoethyl tetra-*O*-acetyl-β-D-glucopyranoside 3[15,18] (14.2 g, 34.2 mmol) was dissolved in anhydrous MeOH (200 mL) and treated at rt with 1 M NaOMe solution in MeOH (10.3 mL). The mixture was stirred at rt for 5 h, neutralized with acid-washed Dowex 50W-X8 ion-exchange resin, filtered, concentrated, and dried under reduced pressure. The crude tetraol was redissolved in THF (80 mL) and treated with *p*-methoxybenzaldehyde dimethyl acetal (9.8 mL, 57.4 mmol) and camphorsulfonic acid (CSA, 476 mg, 2.1 mmol), then heated to reflux for 10 h. The reaction mixture was quenched with saturated aq NaHCO3 (50 mL), extracted with CH_2Cl_2 (3 × 150 mL), dried over Na_2SO_4 and concentrated, then purified by silica gel chromatography (50% EtOAc in hexanes) to afford the 4,6-anisylidene acetal as an amorphous white solid (9.3 g). The resulting 2,3-diol was redissolved in anhydrous DMF (160 mL) and treated at 0 °C with BnBr (5.8 mL, 49.1 mmol) and NaH (1.47 g,

Table 2
Mono- and disaccharide α-GlcN sulfoforms prepared by solid-phase synthesis[15] (overall yields)



36.8 mmol). The mixture was warmed to rt over 2 h and stirred for another 10 h, then quenched with saturated aq NH_4Cl (100 mL), extracted with CH_2Cl_2 (3 × 200 mL), washed with brine (50 mL), dried over Na_2SO_4 and concentrated, then purified by silica gel chromatography (20% EtOAc in hexanes) to afford the 2,3-di-*O*-benzyl ether as an amorphous white solid (7.7 g, 62% over three steps).

The 4,6-anisylidene acetal (7.6 g, 13.8 mmol) was dissolved in THF (274 mL) and treated at −78 °C with a 1 M solution of BH_3 in THF (76.0 mmol) and a 1 M solution of Bu_2BOTf in THF (34.8 mL). The mixture was stirred at −78 °C for 10 h, then quenched with Et_3N (6.8 mL, 48.6 mmol) and MeOH (200 mL), and warmed slowly to rt over 1 h. The product was extracted with CH_2Cl_2 (3 × 200 mL), washed with brine (40 mL), dried over Na_2SO_4, and concentrated, then purified by silica gel chromatography (25% EtOAc in hexanes) to afford 6-*O*-PMB ether **4** as an amorphous white solid (7.5 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35 (m, 12H), 6.89 (d, 2H, J = 8.6 Hz), 4.99 (t, 2H, J = 11.5 Hz), 4.75 (d, 2H, J = 11.3 Hz), 4.52 (m, 2H), 4.46 (d, 1H, J = 7.3 Hz), 4.07 (ddd, 1H, J = 3.8, 5.4, 9.9 Hz), 3.81 (s, 3H), 3.60 (m, 9H), 2.60 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.23, 138.50, 138.31, 129.82, 129.35, 128.49, 128.34, 128.07, 127.90, 127.80, 127.67, 113.76, 103.62, 83.85, 81.63, 75.22, 74.77, 74.03, 73.25, 71.39, 69.77, 68.14, 55.21, 50.96; IR (NaCl): 3460, 2916, 2872, 2104, 1612, 1513, 1248, 1064 cm^{-1}; $[\alpha]_D^{20}$ = –13.2 (*c* 1.21, CH_2Cl_2); HRESI-MS: *m*/*z* calcd for $C_{30}H_{35}N_3NaO_7$ [M+Na]$^+$: 572.2373; found: 572.2375.

3.3. 2′-(*N*-Carboxybenzyl)aminoethyl 2,3-di-*O*-benzyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranoside (5)

A solution of 2′-azidoethyl glucoside **4** (478 mg, 0.87 mmol) in CH_2Cl_2 (20 mL) was treated with Bu_3P (225 μL, 0.91 mmol), and stirred at rt for 3 h, then treated with H_2O (10 mL) and stirred at rt for 1 day. The crude amine was concentrated and dried under reduced pressure with azeotropic distillation with toluene (3 × 10 mL), to afford a yellowish oil. This was redispersed with $NaHCO_3$ (263 mg, 3.10 mmol) in 5:1 THF/H_2O (10 mL), then treated at 0 °C with Cbz-Cl (135 μL, 0.96 mmol). The reaction mixture was warmed to rt over 2 h and stirred for another 10 h, then extracted with CH_2Cl_2 (3 × 50 mL), washed with brine (20 mL), dried over Na_2SO_4, and concentrated, and purified by silica gel chromatography (33% EtOAc in hexanes) to afford Cbz-protected 2′-aminoethyl glucoside **5** as an amorphous white solid (526 mg, 92% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36 (m, 15H), 7.24 (d, 2H, J = 8.2 Hz), 6.88 (d, 2H, J = 8.2 Hz), 5.62 (br, 1H), 5.11 (s, 2H), 4.96 (d, 1H, J = 11.4 Hz), 4.90 (d, 1H, J = 11.1 Hz),4.78 (t, 2H, J = 13.0 Hz), 4.99 (m, 2H), 4.41 (d, 1H, J = 7.1 Hz), 3.87 (m, 6H), 3.63 (m, 2H), 3.46 (m, 5H), 2.84 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.23, 156.53, 138.54, 138.24, 136.56, 129.81, 129.36, 128.49, 128.47, 128.41, 128.11, 128.00, 127.91, 127.80, 127.74, 113.78, 103.83, 84.00, 81.66, 75.23, 74.83, 74.09, 73.16, 71.33, 69.87, 69.61, 66.64, 55.20, 41.42; IR (NaCl): 3360, 2872, 1711, 1513, 1248, 1062 cm^{-1}; $[\alpha]_D^{20}$ = –16.7 (*c* 0.65, CH_2Cl_2); HRESI-MS: *m*/*z* calcd for $C_{38}H_{43}NNaO_9$ [M+Na]$^+$: 680.2836; found: 680.2835.

3.4. Thiotolyl 2-azido-4-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-3-*O*-[2-(trimethylsilyl)ethoxymethyl]-β-D-glucopyranoside (7)

3-*O*-Acetyl derivative **6** (1.0 g, 1.47 mmol) was prepared as previously described,[15] then dissolved in anhydrous MeOH (60 mL) and treated at rt with 1 M NaOMe solution in MeOH (440 μL). The mixture was stirred for 5 h at rt, neutralized with activated Dowex 50X-W-H$^+$ ion-exchange resin, filtered, concentrated, and dried under reduced pressure. The crude C3 alcohol was redissolved in CH_2Cl_2 (40 mL) and treated at rt with TBAI (813 mg, 2.20 mmol) and *i*Pr_2NEt (2.56 mL, 14.70 mmol), then SEM-Cl (1.82 mL, 10.30 mmol). The mixture was stirred for 48 h at rt, then extracted with CH_2Cl_2 (2 × 100 mL), washed with brine (40 mL), dried over Na_2SO_4, then concentrated under reduced pressure. Purification by silica gel chromatography (4.8% EtOAc in hexanes) yielded 3-*O*-SEM ether **7** as an amorphous white solid (1.03 g, 91% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (dd, 2H, J = 1.2, 7.7 Hz), 7.71 (dd, 2H, J = 1.3, 9.1 Hz), 7.55 (d, 2H, J = 8.1 Hz), 7.36 (m, 9H), 7.15 (dd, 2H, J = 3.6, 7.3 Hz), 7.05 (d, 2H, J = 8.0 Hz), 4.98 (d, 1H, J = 6.4 Hz), 4.88 (d, 1H, J = 6.4 Hz), 4.79 (d, 1H, J = 10.8 Hz), 4.78 (d, 1H, J = 8.2 Hz), 4.69 (d, 1H, J = 10.6 Hz), 4.41 (d, 1H, J = 10.1 Hz), 4.01 (dd, 1H, J = 1.4, 11.5 Hz), 3.92 (dd, 1H, J = 3.1, 11.4 Hz), 3.70 (m, 5H), 3.30 (m, 2H), 2.33 (s, 3H), 1.11 (s, 9H), 0.97 (t, 2H, J = 8.3 Hz), 0.00 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.50, 137.72, 135.83, 135.59, 134.05, 133.25, 132.73, 129.76, 128.42, 127.78, 127.71, 127.67, 127.37, 96.51, 86.33, 81.65, 79.91, 74.81, 66.40, 64.57, 62.23, 26.84, 21.15, 19.27, 18.08, –1.51; IR (NaCl): 2952, 2857, 2111, 1428, 1248, 1112, 1049 cm^{-1}; $[\alpha]_D^{20}$ = –21.8 (*c* 1.35, CH_2Cl_2); HRESI-MS: *m*/*z* calcd for $C_{43}H_{55}N_3NaO_5SSi_2$ [M+Na]$^+$: 792.3299; found: 792.3313.

3.5. 2′-(*N*-Carboxybenzyl)aminoethyl 4-*O*-[2-azido-4-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-3-*O*-[2-(trimethylsilyl)ethoxymethyl]-α-D-glucopyranosyl]-2,3-di-*O*-benzyl-6-*O*-*p*-methoxybenzyl-β-D-glucopyranoside (1)

Thioglycoside donor **7** (200 mg, 0.26 mmol) and glucoside acceptor **5** (248 mg, 0.38 mmol) were dissolved in 1:2 $(CH_2Cl)_2$/Et_2O (6 mL) and treated with freshly activated 4A molecular sieves (800 mg) for 1 h at rt under argon. The mixture was then cooled to −30 °C and treated with NIS (64 mg, 0.29 mmol) and TfOH (3.5 μL, 0.04 mmol). The mixture was stirred for 5 h at −30 °C, then quenched with Et_3N (22 μL, 0.16 mmol), and filtered through Celite, which was rinsed with CH_2Cl_2 (3 × 70 mL). The filtrate was washed with brine (40 mL), dried over Na_2SO_4, and concentrated, then purified by silica gel chromatography (20% EtOAc in hexanes) to yield orthogonally protected GlcN(α1→4)Glc disaccharide **1** as an amorphous white solid (229 mg, 68%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (d, 2H, J = 6.7 Hz), 7.64 (d, 2H, J = 7.0 Hz), 7.30 (m, 26H), 7.00 (d, 2H, J = 8.2 Hz), 6.60 (d, 2H, J = 8.3 Hz), 5.70 (d, 1H, J = 3.7 Hz), 5.60 (br, 1H), 5.04 (m, 5H), 4.89 (m, 5H), 4.72 (dd, 2H, J = 5.7, 11.0 Hz), 4.42 (d, 1H, J = 7.9 Hz), 4.29 (m, 2H), 3.73 (m, 18H), 3.06 (dd, 1H, J = 3.8, 7.9 Hz), 1.07 (s, 9H), 1.01 (t, 2H, J = 8.6 Hz), 0.04 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.92, 156.48, 138.43, 138.08, 137.99, 136.54, 135.84, 135.50, 133.55, 132.91, 129.60, 128.95, 128.42, 128.38, 128.11, 128.00, 127.92, 127.67, 127.53, 127.44, 113.53, 103.70, 97.66, 96.47, 84.71, 82.54, 78.11, 74.87, 74.72, 74.56, 74.00, 72.93, 72.18, 70.04, 68.88, 66.60, 66.19, 62.55, 61.86, 55.03, 41.51, 26.83, 19.28, 18.11, −1.51; IR (NaCl): 3032, 2952, 2108, 1724, 1513, 1454, 1360, 1248, 1112, 1040 cm^{-1}; $[\alpha]_D^{20}$ = +55.0 (*c* 1.02, CH_2Cl_2); HRMALDI–MS: *m*/*z* calcd for $C_{73}H_{90}N_4NaO_{14}Si_2$ [M+Na]$^+$: 1325.5889; found: 1325.5785.

3.6. 2′-(*N*-Carboxybenzyl)aminoethyl 4-*O*-[2-azido-4-*O*-benzyl-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-3-*O*-[2-(trimethylsilyl)ethoxymethyl]-α-D-glucopyranosyl]-2,3-di-*O*-benzyl-β-D-glucopyranoside (8)

6-*O*-PMB ether **1** (800 mg, 0.61 mmol) was dissolved in 90% aqueous CH_3CN (8 mL), treated at 0 °C with CAN (1 g, 1.84 mmol), then stirred for 10 h. The mixture was quenched with Et_3N (342 μL, 2.46 mmol), concentrated, and purified by silica gel chromatography (20% EtOAc in hexanes) to afford C6 alcohol **8** as a colorless oil (630 mg, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (dd, 4H, J = 7.1, 8.4 Hz), 7.39 (m, 26H), 5.81 (d, 1H, J = 3.5 Hz), 5.55 (t, 1H,

J = 6.0 Hz), 5.16 (m, 4H), 4.99 (m, 3H), 4.92 (d, 1H, J = 10.8 Hz), 4.79 (d, 2H, J = 11.0 Hz), 4.52 (d, 1H, J = 7.7 Hz), 4.12 (t, 1H, J = 8.7 Hz), 3.13 (m, 13H), 3.47 (m, 5H), 3.20 (dd, 1H, J = 3.6, 10.4 Hz), 2.44 (t, 1H, J = 6.0 Hz), 1.18 (s, 9H), 1.10 (t, 3H, J = 8.4 Hz), 0.11 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.18, 138.30, 137.90, 137.62, 136.22, 135.68, 135.42, 133.23, 132.73, 129.51, 128.25, 127.98, 127.76, 127.60, 127.44, 127.32, 103.58, 97.82, 96.30, 84.43, 82.43, 78.08, 76.46, 74.79, 74.58, 72.42, 72.32, 69.36, 66.55, 66.06, 62.39, 62.18, 61.67, 41.05, 26.72, 19.13, 17.96, −1.60; IR (NaCl): 2952, 2108, 1717, 1456, 1249, 1113, 1041 cm^{-1}; $[\alpha]_D^{20}$ = +53.1 (c 1.02, CH_2Cl_2); ESI–MS: m/z calcd for $C_{65}H_{82}N_4NaO_{13}Si_2$ $[M+Na]^+$: 1205.5; found: 1205.4.

3.7. 2′-(*N*-Carboxybenzyl)aminoethyl 4-*O*-[2-acetamido-4-*O*-benzyl-2-deoxy-6-*O*-sulfonato-α-D-glucopyranosyl]-2,3-di-*O*-benzyl-6-*O*-sulfonato-β-D-glucopyranoside (9a)

Op. 1: Orthogonally protected disaccharide **8** (70 mg, 0.06 mmol) was dissolved in THF (0.85 mL), then treated with a 1 M solution of TBAF in THF (0.35 mL) and stirred at rt for 6 h. The mixture was concentrated then purified by silica gel chromatography (40% EtOAc in hexanes) to yield the C6′ alcohol as a white solid (49 mg, 87%). *Op. 2*: The intermediate azide (40 mg, 0.04 mmol) was dissolved in dry pyridine (0.6 mL), then treated with AcSH (121 μL, 1.69 mmol) and stirred at rt for 48 h. The mixture was concentrated and purified by silica gel chromatography (3% MeOH in $CHCl_3$) to yield the C2′ *N*-acetamide as an amorphous white solid (38 mg, 95%). *Op. 3*: The intermediate diol (34 mg, 0.04 mmol) was dissolved in dry DMF (1.1 mL), then treated with $SO_3{\cdot}Me_3N$ (98 mg, 0.70 mmol) and stirred at 55 °C for 10 h. The reaction mixture was concentrated and purified by silica gel chromatography (5% MeOH in $CHCl_3$ with 2% Et_3N) to yield the 6′,6-di-*O*-sulfate Et_3NH salt as a yellow solid (44 mg, 93%). *Op. 4*: The intermediate SEM ether (20 mg, 0.02 mmol) was dissolved in Et_2O (1 mL), then treated with a solution of $MgBr_2{\cdot}Et_2O$ (46 mg, 0.18 mmol) in CH_3NO_2 (0.9 mL) and Et_2O (0.8 mL) and stirred at rt for 48 h. The reaction mixture was quenched with saturated $NaHCO_3$ (2 mL), extracted with CH_2Cl_2 (3 × 2 mL), washed with brine (2 mL), dried over Na_2SO_4, and concentrated. *Op. 5*: The crude disulfate ester was dissolved in 50% aq MeOH and passed through an ion-exchange column, then concentrated and purified by reverse-phase HPLC to yield the disodium salt as an amorphous white solid (14 mg, 79%). The overall yield of 6′,6-di-*O*-sulfate **9a** (disodium salt) from **1** was 52% over 6 operations. ^{1}H NMR (400 MHz, CD_3OD): δ 7.44 (d, 3H, J = 7.4 Hz), 7.27 (m, 17H), 5.44 (d, 1H, J = 3.9 Hz), 4.98 (d, 1H, J = 5.5 Hz), 4.91 (s, 2H), 4.81 (d, 2H, J = 2.1 Hz), 4.56 (t, 2H, J = 10.4 Hz), 4.50 (d, 1H, J = 7.7 Hz), 4.34 (m, 3H), 4.13 (dd, 1H, J = 5.6, 11.2 Hz), 4.04 (dd, 1H, J = 3.7, 10.7 Hz), 3.92 (m, 2H), 3.70 (m, 6H), 3.56 (t, 1H, J = 9.5 Hz), 3.43 (m, 1H), 1.80 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.82, 158.86, 139.83, 138.25, 129.87, 129.54, 129.39, 129.21, 129.13, 129.08, 128.88, 128.78, 128.66, 128.50, 128.34, 104.46, 99.22, 85.02, 83.56, 79.49, 77.76, 75.82, 75.72, 75.40, 75.27, 74.49, 73.99, 69.37, 67.86, 67.39, 62.01, 54.77, 41.97, 22.97; $[\alpha]_D^{20}$ = +73.6 (c 1.0, MeOH); HRESI-MS: m/z calcd for $C_{45}H_{52}N_2Na_3O_{19}S_2$ $[M+3Na]^+$: 1057.2299; found: 1057.2282.

3.8. 2′-(*N*-Carboxybenzyl)aminoethyl 4-*O*-[2-acetamido-4-*O*-benzyl-2-deoxy-3-*O*-sulfonato-α-D-glucopyranosyl]-2,3-di-*O*-benzyl-6-*O*-sulfonato-β-D-glucopyranoside (9b)

Op. 1: Orthogonally protected disaccharide **8** (85 mg, 0.07 mmol) was dissolved in Et_2O (3 mL), then treated with a solution of $MgBr_2{\cdot}Et_2O$ (185 mg, 0.72 mmol) in CH_3NO_2 (3 mL) and Et_2O (3 mL) and stirred at rt for 48 h. The reaction mixture was quenched with saturated aq $NaHCO_3$ (10 mL), extracted with EtOAc (3 × 15 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated, then purified by silica gel chromatography (40% EtOAc in hexanes) to yield the C3′ alcohol as a white solid (70 mg, 93%). *Op. 2*: The intermediate azide (40 mg, 0.04 mmol) was dissolved in dry pyridine (0.58 mL), then treated with AcSH (0.11 mL) and stirred at rt for 48 h. The mixture was concentrated and purified by silica gel chromatography (3% MeOH in $CHCl_3$) to yield the C2′ *N*-acetamide as an amorphous white solid (38 mg, 93%). *Op. 3*: The intermediate diol (38 mg, 0.04 mmol) was dissolved in dry DMF (1.2 mL), then treated with $SO_3{\cdot}Me_3N$ (99 mg, 0.71 mmol) and stirred at 55 °C for 10 h. The reaction mixture was concentrated and purified by silica gel chromatography (3% MeOH in $CHCl_3$ with 2% Et_3N) to yield the 3′,6-di-*O*-sulfate Et_3NH salt as a yellow solid (44 mg, 93%). *Op. 4*: The intermediate TBDPS ether (45 mg, 0.04 mmol) was dissolved in DMF (0.4 mL), then treated with a 1 M solution of TBAF in THF (0.4 mL) for 48 h and concentrated to obtain a yellow oil. *Op. 5*: The crude disulfate ester was dissolved in 50% aq MeOH and passed through an ion-exchange column, then concentrated, and purified by reverse-phase HPLC to yield the disodium salt as an amorphous white solid (20 mg, 55%). The overall yield of 3′,6-di-*O*-sulfate **9b** (disodium salt) from **1** was 39% over 6 operations. ^{1}H NMR (400 MHz, CD_3OD): δ 7.45 (m, 2H), 7.23 (m, 18H), 5.49 (d, 1H, J = 3.4 Hz), 5.15 (d, 2H, J = 10.3 Hz), 4.99 (d, 2H, J = 7.4 Hz), 4.93 (d, 2H, J = 11.2 Hz), 4.71 (m, 2H), 4.54 (m, 3H), 4.35 (dd, 1H, J = 2.3, 10.7 Hz), 4.23 (dd, 1H, J = 4.7, 10.9 Hz), 4.07 (dd, 1H, J = 3.5, 10.8 Hz), 3.94 (m, 1H), 3.95 (m, 3H), 3.76 (t, 1H, J = 8.6 Hz), 3.69 (dd, 3H, J = 4.1, 12.1 Hz), 3.59 (t, 1H, J = 9.4 Hz), 3.42 (t, 1H, J = 8.3 Hz), 3.36 (dd, 2H, J = 5.3, 11.0 Hz), 1.76 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ 173.40, 158.86, 140.07, 139.84, 139.29, 138.24, 129.39, 129.28, 129.17, 129.07, 128.96, 128.87, 128.77, 128.64, 128.57, 128.39, 104.53, 98.67, 85.17, 83.54, 79.33, 75.86, 75.48, 75.26, 74.48, 74.38, 73.36, 72.00, 69.40, 68.09, 67.58, 67.39, 55.11, 41.95, 22.64; $[\alpha]_D^{20}$ = +78.9 (c 1.0, MeOH); HRESI-MS: m/z calcd for $C_{45}H_{52}N_2Na_3O_{19}S_2$ $[M+3Na]^+$: 1057.2299; found: 1057.2288.

3.9. 2′-(*N*-Carboxybenzyl)aminoethyl 4-*O*-[2-amino-4-*O*-benzyl-2-deoxy-2-*N*-sulfonato-α-D-glucopyranosyl]-2,3-di-*O*-benzyl-6-*O*-sulfonato-β-D-glucopyranoside (9c)

Op. 1: Orthogonally protected disaccharide **8** (40 mg, 0.03 mmol) was dissolved in dry DMF (1.13 mL), then treated with $SO_3{\cdot}Me_3N$ (98 mg, 0.34 mmol) at 55 °C for 10 h. The mixture was concentrated and purified by silica gel chromatography (2% MeOH in $CHCl_3$ with 2% Et_3N) to yield the 6-*O*-sulfate Et_3NH salt as a yellow solid (40 mg, 90%). *Op. 2*: The intermediate sulfoform (40 mg, 0.03 mmol) was dissolved in Et_2O (2 mL), then treated with $MgBr_2{\cdot}Et_2O$ (80 mg, 0.30 mol) in CH_3NO_2 (1.5 mL) and Et_2O (1 mL) and stirred at rt for 24 h. The mixture was quenched with saturated $NaHCO_3$ (10 mL), extracted with CH_2Cl_2 (3 × 15 mL), washed with brine (10 mL), dried over Na_2SO_4, and concentrated, then purified by silica gel chromatography (5% MeOH in $CHCl_3$ with 2% Et_3N) to yield the C3′ alcohol as a yellow solid (40 mg, 90%). *Op. 3*: The intermediate TBDPS ether (30 mg, 0.02 mmol) was dissolved in DMF (1 mL), then treated with a 1 M solution of TBAF in THF (0.19 mL) and stirred at rt for 24 h. The mixture was concentrated and purified by silica gel chromatography (9% MeOH in $CHCl_3$ with 2% Et_3N) to yield the C6′ alcohol as a yellow solid (19 mg, 78%). *Op. 4*: The intermediate azide (28 mg, 0.03 mmol) was dissolved in MeOH (1 mL), then treated with 1,3-propanedithiol (130 μL, 0.93 mmol) and Et_3N (94 μL, 0.93 mmol) and stirred at rt for 24 h. The mixture was concentrated and purified by silica gel chromatography (10% MeOH in $CHCl_3$ with 2% Et_3N) to yield the C2′ amine as a yellow solid (25 mg, 93%). *Op. 5*: The amine (48 mg, 0.56 mmol) was dissolved

in DMF (1 mL) and mixed with aq NaOH adjusted to pH 9.5 (5 mL), then treated with SO_3·Py (162 mg, 5.60 mmol), stirred at rt for 8 h, and concentrated. *Op. 6*: The crude di-*N*,*O*-sulfate ester was dissolved in 50% aq MeOH and passed through an ion-exchange column, then concentrated, and purified by reverse-phase HPLC to yield the disodium salt as an amorphous white solid (33 mg, 63%). The overall yield of 2′,6-di-*N*,*O*-sulfate **9c** (disodium salt) from **1** was 36% over 7 operations. ^{1}H NMR (400 MHz, CD_3OD): δ 7.07–7.44 (m, 20H), 5.52 (d, 1H, J = 3.5 Hz), 4.99 (d, 1H, J = 3.9 Hz), 4.95 (d, 1H, J = 4.6 Hz), 4.82–4.93 (m, 2H), 4.64 (d, 1H, J = 11.0 Hz), 4.56 (d, 1H, J = 11.2 Hz), 4.49 (d, 1H, J = 7.5 Hz), 4.38 (dd, 1H, J = 2.6, 10.9 Hz), 4.16 (dd, 1H, J = 5.3, 10.7 Hz), 3.81–3.98 (m, 3H), 3.58–3.80 (m, 7H), 3.42 (m, 2H), 3.32–3.37 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 158.84, 140.23, 139.87, 139.55, 138.25, 129.64, 129.53, 129.40, 129.25, 129.14, 129.03, 128.88, 128.78, 128.50, 128.41, 104.50, 99.48, 83.41, 79.42, 75.74, 75.19, 75.15, 75.04, 74.94, 74.43, 73.28, 69.41, 68.03, 67.40, 62.26, 60.15, 41.97; $[\alpha]_D^{20}$ = +53.2 (*c* 0.2, MeOH); HRESI-MS: *m*/*z* calcd for $C_{43}H_{50}N_2Na_3O_{18}S_2$ $[M+3Na]^+$: 1015.2193; found: 1057.2209.

3.10. 2′-(*N*-Carboxybenzyl)aminoethyl 4-*O*-[2-acetamido-4-*O*-benzyl-2-deoxy-3,6-di-*O*-sulfonato-α-D-glucopyranosyl]-2,3-di-*O*-benzyl-6-*O*-sulfonato-β-D-glucopyranoside (9d)

Op. 1: Orthogonally protected disaccharide **8** was treated with TBAF in THF to yield the C6′ alcohol, as described in the synthesis of **9a**. *Op. 2*: The intermediate SEM ether (160 mg, 0.17 mmol) was dissolved in Et_2O (7 mL), then treated with a solution of $MgBr_2$·Et_2O (437 mg, 1.69 mmol) and CH_3NO_2 (182 μL, 3.38 mmol) in Et_2O (5 mL) and stirred at rt for 10 h. The mixture was quenched with saturated aq $NaHCO_3$ (70 mL), extracted with EtOAc (3 × 100 mL), washed with brine (20 mL), dried over Na_2SO_4, and concentrated, then purified by silica gel chromatography (66% EtOAc in hexanes) to yield the C3′ alcohol as an amorphous white solid (134 mg, 97%). *Op. 3*: The intermediate azide (67 mg, 0.08 mmol) was dissolved in pyridine (2 mL), then treated with AcSH (234 μL, 3.29 mmol) and stirred at rt for 40 h. The mixture was concentrated and purified by silica gel chromatography (4.75% MeOH in CH_2Cl_2) to yield the C2′ *N*-acetamide as an amorphous white solid (60 mg, 88%). *Op. 4*: The intermediate amino alcohol (44 mg, 0.05 mmol) was dissolved in 5:1 Py/Et_3N (3 mL) and treated with SO_3·Py (228 mg, 1.43 mmol), then heated to 70 °C under microwave conditions for 1 h. The mixture was concentrated and purified by silica gel chromatography (9% MeOH in CH_2Cl_2 with 2% Et_3N) to yield the 3′,6′,6-tri-*O*-sulfate Et_3NH salt as a yellow solid. *Op. 5*: The crude trisulfate ester was dissolved in 50% aq MeOH and passed through an ion-exchange column, then concentrated and purified by reverse-phase HPLC to yield the trisodium salt as an amorphous white solid (45 mg, 74%). The overall yield of 3′,6′,6-tri-*O*-sulfate **9d** (trisodium salt) from **1** was 48% over 6 operations. ^{1}H NMR (500 MHz, CD_3OD): δ 7.56 (d, 2H, J = 7.2 Hz), 7.16–7.34 (m, 18H), 5.54 (d, 1H, J = 2.0 Hz), 5.12 (d, 1H, J = 9.6 Hz), 4.99 (m, 2H), 4.92 (d, 1H, J = 11.2 Hz), 4.68 (m, 3H), 4.54 (d, 1H, J = 12.0 Hz), 4.52 (d, 1H, J = 8.3 Hz), 4.40 (d, 1H, J = 10.0 Hz), 4.35 (m, 2H), 4.22 (dd, 1H, J = 4.0, 10.6 Hz), 4.11 (dd, 1H, J = 2.4, 8.4 Hz), 3.91–4.02 (m, 2H), 3.85 (t, 1H, J = 8.8 Hz), 3.78 (t, 1H, J = 8.4 Hz), 3.69 (m, 7H), 3.56 (t, 1H, J = 4.4 Hz), 3.42 (t, 1H, J = 7.9 Hz), 3.37 (br, 1H), 1.94 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 173.98, 159.04, 139.99, 139.94, 139.77, 138.40, 130.70, 129.57, 129.40, 129.30, 129.19, 129.07, 128.95, 128.74, 128.51, 104.63, 99.03, 85.21, 83.71, 79.60, 77.58, 76.19, 75.59, 75.28, 75.24, 74.45, 73.59, 72.25, 69.57, 68.05, 67.56, 67.49, 62.32, 55.26, 54.68, 42.12, 23.18; $[\alpha]_D^{20}$ = +25.0 (*c* 0.79, MeOH); ESI–MS: *m*/*z* calcd for $C_{45}H_{51}N_2Na_4O_{22}S_3$ $[M+4Na]^+$: 1159.2; found: 1159.1.

3.11. 2′-(*N*-Carboxybenzyl)aminoethyl 4-*O*-[2-amino-4-*O*-benzyl-2-deoxy-2,6-di-*N*,*O*-sulfonato-α-D-glucopyranosyl]-2,3-di-*O*-benzyl-6-*O*-sulfonato-β-D-glucopyranoside (9e)

Op. 1: Orthogonally protected disaccharide **8** was treated with TBAF in THF to yield the C6′ alcohol, as described in the synthesis of **9a**. *Op. 2*: The intermediate azide (80 mg, 0.09 mmol) was dissolved in MeOH (3 mL), treated with 1,3-propanedithiol (84 μL, 0.85 mmol) and Et_3N (118 μL, 0.85 mmol), then stirred at rt for 40 h. The mixture was concentrated and purified by silica gel chromatography (3.25% MeOH in CH_2Cl_2) to afford the C2 amine as an amorphous white solid (68 mg, 87%). *Op. 3*: The intermediate amino diol (60 mg, 0.07 mmol) was dissolved in 5:1 Py/Et_3N (3 mL) and treated with SO_3·Py (281 mg, 1.76 mmol), then heated to 70 °C under microwave conditions for 1 h. The mixture was concentrated and purified by silica gel chromatography (9% MeOH in CH_2Cl_2 with 2% Et_3N) to yield the 2′,6′,6-tri-*N*,*O*,*O*-sulfate Et_3NH salt as a light yellow oil. *Op. 4*: The intermediate SEM ether was dispersed in Et_2O (4 mL), treated with a solution of $MgBr_2$·Et_2O (169 mg, 0.65 mmol) in CH_3NO_2 (3 mL, 55.80 mmol) in Et_2O (2 mL), then stirred at rt for 40 h. The mixture was concentrated and used directly in the next operation. *Op. 5*: The crude trisulfate ester was dissolved in 50% aq MeOH and passed through an ion-exchange column, then purified by reverse-phase HPLC to yield the trisodium salt as an amorphous white solid (46 mg, 64% over 2 steps). The overall yield of 2′,6′,6-tri-*N*,*O*,*O*-sulfate **9e** (trisodium salt) from **1** was 42% over 6 operations. ^{1}H NMR (500 MHz, CD_3OD): δ 7.45 (d, 2H, J = 7.1 Hz), 7.36 (d, 2H, J = 7.2 Hz), 7.15–7.32 (m, 16H), 5.57 (d, 1H, J = 3.1 Hz), 4.91–5.03 (m, 4H), 4.83 (d, 1H, J = 11.2 Hz), 4.77 (d, 1H, J = 10.5 Hz), 4.58 (d, 1H, J = 13.2 Hz), 4.56 (d, 1H, J = 11.2 Hz), 4.50 (d, 1H, J = 7.3 Hz), 4.27–4.40 (m, 3H), 4.20 (dd, 1H, J = 5.0, 10.8 Hz), 3.84–3.98 (m, 3H), 3.73–3.83 (m, 2H), 3.67 (m, 2H), 3.57 (m, 2H), 3.44 (t, 1H, J = 7.7 Hz), 3.33–3.40 (m, 3H), 1.94 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ 158.99, 140.29, 139.99, 139.82, 138.36, 129.62, 129.58, 129.38, 129.29, 129.24, 129.06, 128.94, 128.67, 128.57, 128.50, 104.64, 99.27, 83.78, 83.43, 79.24, 75.76, 75.35, 75.29, 75.00, 74.60, 71.47, 69.59, 68.15, 67.72, 67.55, 60.06, 47.99, 42.10; $[\alpha]_D^{20}$ = +33.6 (*c* 0.93, MeOH); ESI–MS: *m*/*z* calcd for $C_{43}H_{49}N_2Na_4O_{21}S_3$ $[M+4Na]^+$: 1117.2; found: 1117.0.

3.12. 2′-(*N*-Carboxybenzyl)aminoethyl 4-*O*-[2-amino-4-*O*-benzyl-2-deoxy-2,3-di-*N*,*O*-sulfonato-α-D-glucopyranosyl]-2,3-di-*O*-benzyl-6-*O*-sulfonato-β-D-glucopyranoside (9f)

Op. 1: Orthogonally protected disaccharide **8** (158 mg, 0.13 mmol) was dissolved in Et_2O (20 mL), then treated with a solution of $MgBr_2$·Et_2O (441 mg, 1.34 mmol) and CH_3NO_2 (143 μL, 2.68 mmol) in Et_2O (6 mL) and stirred for 10 h. The mixture was quenched with saturated aq $NaHCO_3$ (70 mL), extracted with EtOAc (3 × 100 mL), washed with brine (20 mL), dried over Na_2SO_4, and concentrated, then purified by silica gel chromatography (66% EtOAc in hexanes) to afford an amorphous white solid (136 mg, 96%). *Op. 2*: The intermediate azide (136 mg, 0.13 mmol) was dissolved in MeOH (3 mL), then treated with 1,3-propanedithiol (133 μL, 1.30 mmol) and Et_3N (186 μL, 1.30 mmol) and stirred at rt for 40 h. The mixture was concentrated and purified by silica gel chromatography (4.75% MeOH in CH_2Cl_2) to afford the C2′ amine as an amorphous white solid (101 mg, 77%). *Op. 3*: A solution of amino diol (93 mg, 0.09 mmol) was dissolved in 5:1 Py/Et_3N (3 mL) and treated with SO_3·Py (385 mg, 2.42 mmol), then heated to 70 °C under microwave conditions for 1 h. The mixture was concentrated and purified by silica gel chromatography (9% MeOH in CH_2Cl_2 with 2% Et_3N) to yield 2′,3′,6-tri-*N*,*O*,*O*-sulfate Et_3NH salt as a light yellow oil. *Op. 4*: The intermediate TBDPS ether was redissolved in DMF (2 mL), then treated with 1 M TBAF

solution in THF (0.9 mL) and stirred at rt for 40 h. The mixture was concentrated and used directly in the next operation. *Op. 5*: The crude trisulfate ester was dissolved in 50% aq MeOH and passed through an ion-exchange column, then purified by reverse-phase HPLC to yield the trisodium salt as an amorphous white solid (61 mg, 62%). The overall yield of 2′,3′,6-tri-*N*,*O*,*O*-sulfate **9f** (trisodium salt) from **1** was 40% over 6 operations. ^{1}H NMR (400 MHz, CD_3OD): δ 7.46 (dd, 2H, J = 1.4, 8.3 Hz), 7.11–7.42 (m, 18H), 5.75 (d, 1H, J = 3.4 Hz), 5.14 (d, 1H, J = 10.8 Hz), 5.10 (d, 1H, J = 11.1 Hz), 4.99 (m, 2H), 4.82 (d, 1H, J = 11.2 Hz), 4.65 (dd, 1H, J = 8.9, 10.5 Hz), 4.58 (d, 1H, J = 10.6 Hz), 4.54 (d, 1H, J = 11.2 Hz), 4.52 (d, 1H, J = 7.3 Hz), 4.37 (dd, 1H, J = 2.0, 10.8 Hz), 4.24 (dd, 1H, J = 4.8, 10.7 Hz), 3.53–4.05 (m, 10H), 3.49 (dd, 1H, J = 3.4, 10.6 Hz), 3.33–3.44 (m, 3H), 1.94 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 158.86, 140.36, 139.98, 139.74, 138.26, 129.93, 129.40, 129.33, 129.17, 129.07, 128.97, 128.90, 128.75, 128.59, 128.40, 128.06, 104.35, 98.36, 84.57, 83.58, 79.47, 78.25, 75.64, 75.19, 74.95, 74.51, 74.15, 73.66, 69.30, 68.13, 67.38, 62.18, 58.86, 41.97; $[\alpha]_D^{20}$ = +54.4 (c 0.62, MeOH); ESI–MS: m/z calcd for $C_{43}H_{49}N_2Na_4O_{21}S_3$ $[M+4Na]^+$: 1117.2; found: 1117.0.

3.13. 2′-(*N*-Carboxybenzyl)aminoethyl 4-*O*-[2-amino-4-*O*-benzyl-2-deoxy-2,3,6-tri-*N*,*O*,*O*-sulfonato-α-D-glucopyranosyl]-2,3-di-*O*-benzyl-6-*O*-sulfonato-β-D-glucopyranoside (9g)

Op. 1: Orthogonally protected disaccharide **8** was treated with TBAF in THF to yield the C6' alcohol, as described in the synthesis of **9a**. *Op. 2*: The intermediate SEM ether was treated with $MgBr_2{\cdot}Et_2O$ and CH_3NO_2 in Et_2O to yield the C3′ alcohol, as described in the synthesis of **9d**. *Op. 3*: The intermediate azide (68 mg, 0.08 mmol) was dissolved in MeOH (3 mL), then treated with 1,3-propanedithiol (101 μL, 1.67 mmol) and Et_3N (232 μL, 1.67 mmol) and stirred at rt for 20 h. The mixture was concentrated and purified by silica gel chromatography (4.75% MeOH in CH_2Cl_2) to yield the C2' amine as an amorphous white solid (62 mg, 94%). *Op. 4*: A solution of amino triol (76 mg, 0.10 mmol) was dissolved in 5:1 Py:Et_3N (3 mL) and treated with $SO_3{\cdot}Py$ (552 mg, 3.47 mmol), then heated to 70 °C under microwave conditions for 1 h. The mixture was concentrated and purified by silica gel chromatography (9% MeOH in CH_2Cl_2 with 2% Et_3N) to yield 2′,3′,6′,6-tetra-*N*,*O*,*O*,*O*-sulfate Et_3NH salt as a light yellow oil. *Op. 4*: The tetrasulfate ester was dissolved in 50% aq MeOH and passed through an ion-exchange column, then purified by reverse-phase HPLC to yield the tetrasodium salt as an amorphous white solid (90 mg, 78% over 2 steps). The overall yield of 2′,3′,6′,6-tetra-*N*,*O*,*O*,*O*-sulfate 9 g (tetrasodium salt) from 1 was 54% over 6 operations.^{1}H NMR (400 MHz, CD_3OD): 7.57 (d, 2H, J = 7.3 Hz), 7.06–7.49 (m, 18H), 5.51 (d, 1H, J = 2.6 Hz), 5.13 (d, 1H, J = 9.5 Hz), 5.06 (d, 1H, J = 11.2 Hz), 4.98 (m, 2H), 4.89 (d, 1H, J = 11.7 Hz), 4.70 (d, 1H, J = 9.5 Hz), 4.63 (t, 1H, J = 9.8 Hz), 4.46–4.60 (m, 3H), 4.23–4.42 (m, 4H), 3.84–4.06 (m, 4H), 3.49–3.84 (m, 6H), 3.41 (t, 1H, J = 8.0 Hz), 3.33 (br, 1H); ^{13}C NMR (125 MHz, CD_3OD): 157.34, 138.84, 138.38, 137.93, 136.68, 129.12, 127.94, 127.73, 127.68, 127.61, 127.55, 127.53, 127.45, 127.31, 127.24, 127.00, 126.58, 102.88, 96.62, 82.75, 82.05, 78.36, 76.26, 74.55, 73.84, 73.07, 72.37, 71.79, 70.22, 67.93, 66.74, 65.95, 57.07, 40.42; $[\alpha]_D^{20}$ = +71.9 (c 0.98, MeOH); ESI–MS: m/z calcd for $C_{43}H_{48}N_2Na_5O_{24}S_4$ $[M+5Na]^+$: 1219.1; found: 1219.1.

3.14. 2′-Aminoethyl 4-*O*-[2-acetamido-2-deoxy-6-*O*-sulfonato-α-D-glucopyranosyl]-6-*O*-sulfonato-β-D-glucopyranoside (2a)

Partially protected sulfoform **9a** (20 mg, 0.02 mmol) was dissolved in 50% aq MeOH (1 mL), then treated with 10% $Pd(OH)_2$ on charcoal (8 mg) and stirred under positive H_2 pressure at rt for 20 h. The product was filtered, concentrated, and purified by reverse-phase HPLC to yield 6′,6-di-*O*-sulfate **2a** (disodium salt) as an amorphous white solid (10.5 mg, 87%). ^{1}H NMR (400 MHz, D_2O): δ 5.38 (d, 1H, J = 3.6 Hz) 4.42 (d, 1H, J = 8.0 Hz), 4.26 (m, 4H), 4.15 (dd, 1H, J = 5.1, 11.4 Hz), 3.88 (d, 2H, J = 3.6 Hz), 3.84 (m, 3H), 3.73 (m, 1H) 3.68 (m, 1H) 3.63 (t, 2H, J = 8.8 Hz), 3.54 (t, 1H, J = 9.5 Hz), 3.24 (m, 1H), 2.98 (t, 1H, J = 5.2 Hz), 1.96 (s, 3H); ^{13}C NMR (100 MHz, D_2O): δ 174.98, 102.66, 98.02, 76.91, 75.37, 73.93, 73.03, 71.31, 71.17, 69.71, 69.47, 67.74, 67.16, 54.20, 40.42, 22.53; $[\alpha]_D^{20}$ = +56.0 (c 0.5, H_2O); HRESI-MS: m/z calcd for $C_{16}H_{28}N_2NaO_{17}S_2$ $[M+Na]^-$: 607.0727; found: 607.0733.

3.15. 2′-Aminoethyl 4-*O*-[2-acetamido-2-deoxy-3-*O*-sulfonato-α-D-glucopyranosyl]-6-*O*-sulfonato-β-D-glucopyranoside (2b)

Partially protected sulfoform **9b** (20 mg, 0.02 mmol) was dissolved in 50% aq MeOH (1 mL), then treated with 10% $Pd(OH)_2$ on charcoal (8 mg) and stirred under positive H_2 pressure at rt for 20 h. The product was filtered, concentrated, and purified by reverse-phase HPLC to yield 3′,6-di-*O*-sulfate **2b** (disodium salt) as an amorphous white solid (10 mg, 83%). ^{1}H NMR (400 MHz, D_2O): δ 5.30 (d, 1H, J = 3.7 Hz), 4.44 (m, 1H), 4.36 (m, 1H), 4.29 (dd, 1H, J = 2.1, 11.3 Hz), 4.18 (dd, 1H, J = 4.5, 11.2 Hz), 4.01 (dd, 1H, J = 3.6, 10.8 Hz), 3.90 (dd, 1H, J = 5.4, 11.0 Hz), 3.78 (dd, 2H, J = 2.9, 7.4 Hz), 3.70 (m, 3H), 3.62 (m, 3H), 3.22 (m, 1H) 2.93 (t, 1H, J = 5.3 Hz), 1.93 (s, 3H); ^{13}C NMR (100 MHz, D_2O): δ 174.91, 102.69, 99.28, 80.18, 76.80, 76.58, 73.93, 73.23, 73.08, 70.09, 68.64, 67.52, 60.64, 53.10, 40.49, 22.73; $[\alpha]_D^{20}$ = +31.1 (c 0.45, H_2O); HRESI-MS: m/z calcd for $C_{16}H_{28}N_2NaO_{17}S_2$ $[M+Na]^-$: 607.0727; found: 607.0722.

3.16. 2′-Aminoethyl 4-*O*-[2-amino-2-deoxy-2-*N*-sulfonato-α-D-glucopyranosyl]-6-*O*-sulfonato-β-D-glucopyranoside (2c)

Partially protected sulfoform **9c** (20 mg, 0.02 mmol) was dissolved in 50% aq MeOH (1 mL), then treated with 10% $Pd(OH)_2$ on charcoal (8 mg) and stirred under positive H_2 pressure at rt for 20 h. The product was filtered, concentrated, and purified by reverse-phase HPLC to yield 2′,6-di-*N*,*O*-sulfate **2c** (disodium salt) as an amorphous white solid (10 mg, 84%). ^{1}H NMR (400 MHz, D_2O): δ 5.50 (d, 1H, J = 3.5 Hz), 4.44 (d, 1H, J = 8.1 Hz), 4.28 (dd, 1H, J = 1.9, 11.2 Hz), 4.13 (dd, 1H, J = 4.9, 11.5 Hz), 3.97 (m, 1H), 3.89 (m, 1H), 3.78 (dd, 1H, J = 2.4, 12.6 Hz), 3.71 (m, 3H), 3.66 (m, 2H), 3.52 (t, 1H, J = 9.5 Hz), 3.43 (t, 1H, J = 9.5 Hz), 3.27 (m, 1H), 3.14 (m, 3H); ^{13}C NMR (100 MHz, D_2O): δ 102.57, 98.98, 76.54, 76.15, 73.35, 73.10, 73.04, 71.74, 70.10, 67.60, 67.11, 60.80, 58.72, 40.13; $[\alpha]_D^{20}$ = +26.2 (c 0.8, H_2O); HRESI-MS: m/z calcd for $C_{14}H_{26}N_2NaO_{16}S_2$ $[M+Na]^-$: 565.0622; found: 565.0613.

3.17. 2′-Aminoethyl 4-*O*-[2-acetamido-2-deoxy-3,6-di-*O*-sulfonato-α-D-glucopyranosyl]-6-*O*-sulfonato-β-D-glucopyranoside (2d)

Partially protected sulfoform **9d** (20.3 mg, 0.02 mmol) was dissolved in 50% aq MeOH (3 mL), then treated with 10% $Pd(OH)_2$ on charcoal (20 mg) and stirred under positive H_2 pressure at rt for 20 h. The product was filtered, concentrated, and purified by reverse-phase HPLC to yield 3′,6′,6-tri-*O*-sulfate **2d** (trisodium salt) as an amorphous white solid (12.7 mg, 97%). ^{1}H NMR (400 MHz, D_2O): δ 5.32 (d, 1H, J = 3.6 Hz), 4.43 (d, 1H, J = 8.0 Hz), 4.36 (t, 1H, J = 10.4 Hz), 4.15–4.30 (m, 5H), 4.02 (dd, 1H, J = 3.6, 10.7 Hz), 3.86–3.98 (m, 3H), 3.70–3.77 (m, 1H), 3.59–3.69 (m, 3H), 3.25 (t, 1H, J = 8.4 Hz), 3.08–3.21 (m, 2H), 1.92 (s, 3H); ^{13}C NMR (125 MHz, D_2O): δ 174.21, 101.86, 98.17, 79.24, 75.83, 75.58, 73.11, 72.36, 70.69, 67.59, 66.84, 66.42, 66.12, 52.27, 39.42, 22.04; $[\alpha]_D^{20}$ = +39.2 (c 0.85, H_2O); HRESI-MS: m/z calcd for $C_{16}H_{27}N_2Na_4O_{20}S_3$ $[M+4Na]^+$: 754.9910; found: 754.9918.

3.18. 2′-Aminoethyl 4-*O*-[2-acetamido-2-deoxy-2,6-di-*O*-sulfonato-α-D-glucopyranosyl]-6-*O*-sulfonato-β-D-glucopyranoside (2e)

Partially protected sulfoform **9e** (24.4 mg, 0.02 mmol) was dissolved in 50% aq MeOH (3 mL), then treated with 10% $Pd(OH)_2$ on charcoal (24 mg) and stirred under positive H_2 pressure at rt for 20 h. The product was filtered, concentrated, and purified by reverse-phase HPLC to yield 2′,6′,6-tri-*N*,*O*,*O*-sulfate **2e** (trisodium salt) as an amorphous white solid (15.1 mg, 98%). ^{1}H NMR (400 MHz, D_2O): δ 5.65 (d, 1H, J = 3.6 Hz), 4.56 (t, 1H, J = 7.7 Hz), 4.31–4.38 (m, 3H), 4.25 (dd, 1H, J = 4.9, 11.4 Hz), 4.00–4.19 (m, 2H), 3.80–3.90 (m, 3H), 3.74 (t, 1H, J = 9.1 Hz), 3.61 (m, 2H), 3.34–3.48 (m, 2H), 3.30 (m, 2H), 2.91 (s, 2H), 2.76 (s, 2H); ^{13}C NMR (125 MHz, D_2O): δ 101.90, 97.94, 75.87, 75.06, 72.62, 72.40, 70.98, 70.44, 69.01, 66.96, 66.53, 64.89, 57.89, 42.80; $[\alpha]_D^{20}$ = +48.4 (c 1.0, H_2O); HRESI-MS: m/z calcd for $C_{14}H_{25}N_2Na_4O_{19}S_3$ $[M+4Na]^+$: 712.9804; found: 712.9809.

3.19. 2′-Aminoethyl 4-*O*-[2-amino-2-deoxy-2,3-di-*N*,*O*-sulfonato-α-D-glucopyranosyl]-6-*O*-sulfonato-β-D-glucopyranoside (2f)

Partially protected sulfoform **9f** (8.8 mg, 0.01 mmol) was dissolved in 50% aq MeOH (3 mL), then treated with 10% $Pd(OH)_2$ on charcoal (9 mg) and stirred under positive H_2 pressure at rt for 20 h. The product was filtered, concentrated, and purified by reverse-phase HPLC to yield 2′,3′,6-tri-*N*,*O*,*O*-sulfate **2f** (trisodium salt) as an amorphous white solid (5.4 mg, 97%). ^{1}H NMR (400 MHz, D_2O): δ 5.46 (d, 1H, J = 2.9 Hz), 4.46 (t, 1H, J = 8.0 Hz), 4.28 (m, 2H), 4.13 (dd, 1H, J = 4.8, 11.3 Hz), 3.90–4.07 (m, 2H), 3.66–3.86 (m, 6H), 3.61 (t, 2H, J = 9.2 Hz), 3.26–3.37 (m, 3H), 3.11–3.21 (m, 1H), 2.86 (s, 2H), 2.66 (s, 1H); ^{13}C NMR (125 MHz, D_2O): δ 101.93, 99.19, 78.22, 75.77, 72.45, 72.28, 68.08, 66.88, 64.86, 63.40, 59.94, 57.00, 42.73; $[\alpha]_D^{20}$ = +56.7 (c 0.36, H_2O); HRESI-MS: m/z calcd for $C_{14}H_{25}N_2Na_4O_{19}S_3$ $[M+4Na]^+$: 712.9804; found: 712.9794.

3.20. 2′-Aminoethyl 4-*O*-[2-amino-2-deoxy-2,3,6-tri-*N*,*O*,*O*-sulfonato-α-D-glucopyranosyl]-6-*O*-sulfonato-β-D-glucopyranoside (2g)

Partially protected sulfoform **9g** (47.9 mg, 0.04 mmol) was dissolved in 50% aq MeOH (3 mL), then treated with 10% $Pd(OH)_2$ on charcoal (24 mg) and stirred under positive H_2 pressure at rt for 20 h. The product was filtered, concentrated, and purified by reverse-phase HPLC to yield 2′,3′,6′,6-tetra-*N*,*O*,*O*,*O*-sulfate **2g** (tetrasodium salt) as an amorphous white solid (31.4 mg, 99%). ^{1}H NMR (400 MHz, D_2O): δ 5.55 (d, 1H, J = 3.0 Hz), 4.59 (d, 1H, J = 8.0 Hz), 4.25–4.44 (m, 5H), 3.97–4.18 (m, 3H), 3.68–3.96 (m, 5H), 3.16–3.50 (m, 4H); ^{13}C NMR (125 MHz, D_2O): δ 101.94, 98.75, 81.70, 78.10, 76.52, 75.79, 72.49, 72.29, 70.56, 67.73, 66.90, 66.32, 55.87, 39.50; $[\alpha]_D^{20}$ = +34.6 (c 1.0, H_2O); HRESI-MS: m/z calcd for $C_{14}H_{24}N_2Na_5O_{22}S_4$ $[M+5Na]^+$: 814.9192; found: 814.9194.

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